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Autonomous Pathogen Detection System FY 02 Annual Progress Report

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Autonomous Pathogen Detection System

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Objective

The objective of this project is to design, fabricate and field demonstrate a biological agent detection and identification capability, the Autonomous Pathogen Detector System (APDS). Integrating a flow cytometer and real-time polymerase chain reaction (PCR) detector with sample collection, sample preparation and fluidics will provide a compact, autonomously operating instrument capable of simultaneously detecting multiple pathogens and/or toxins. The APDS will operate in fixed locations, continuously monitoring air samples and automatically reporting the presence of specific biological agents. The APDS will utilize both multiplex immunoassays and nucleic acid assays to provide "quasi-orthogonal" multiple agent detection approaches to minimize false positives and increase the reliability of identification. Technical advances across several fronts must occur, however, to realize the full extent of the APDS. The end goal of a commercially available system for civilian biological weapon defense will be accomplished through three progressive generations of APDS instruments. The APDS is targeted for civilian applications in which the public is at high risk of exposure to covert releases of bioagent, such as major subway systems and other transportation terminals, large office complexes and convention centers. APDS is also designed to be part of a monitoring network of sensors integrated with command and control systems for wide-area monitoring of urban areas and major public gatherings. In this latter application there is potential that a fully developed APDS could add value to DoD monitoring architectures.

The top-level goals of the program are to:

- Develop and field demonstrate an autonomous system utilizing a single-agent flow cytometer assay
- Add multi-agent analysis capability (multiplex detection)
- Add PCR identification capability
- Commercialize the technology
- Partner with first-responder agencies

Recent Progress

The initial plan for APDS in FY02, under the current funding, was to demonstrate its capabilities at a major public event. Given the envisioned development track, this demonstration was planned as an operational test using only simulant assays. The anthrax releases in the fall of 2002, however, shifted the emphasis to rapid deployment of a real agent system. The goal was to develop and field test a fully functional system and begin transferring the technology to a commercial partner as soon as possible. Although laboratory tests showing successful integration of the flow-through PCR module were conducted in FY01, there remained many outstanding issues related to sample preparation (spore lysis, removal of PCR inhibitors, reagent lifetime, etc.) and assays that precluded it from being fielded in a functional capacity in FY02. Given these issues and the compressed timeline, full integration of the PCR component was delayed and the first real agent, automated multiplexed immunoassay pathogen detection system was deployed at Dugway Proving Grounds in September of 2002.

Overview

The current automated pathogen detection system (ADPS-II) is capable of collecting an aerosol sample, performing all sample preparation steps (i.e. mix, wash, incubation, etc.), and performing multiplex detection using a Luminex flow cytometer. The aerosol collector continuously samples the air and traps particles in a swirling water solution. Particles of a given size distribution can be selected by varying the flow rate across a virtual impactor unit. At given time intervals, the collected sample is added to optically encoded microbeads. Each color of microbead contains a capture assay that is specific for a given bioagent. Fluorescent labels are then added to identify the presence of each agent on the bound bead. Each optically encoded and fluorescently labeled microbead is individually read in the flow cytometer, and fluorescent intensities are then correleated with bioagent concentrations. A second detection system has been developed, but not integrated, for confirmation based on nucleic acid amplification and detection. An archived sample is mixed with the Tawman reagent, and then introduced by the SIA system into the flow-through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified and detected using fluorescence generated from nucleic acid replication from the Taqman probes.

Real agent assay development

Assays developed on the APDS platform would ideally be validated by an external, unbiased source. To this end, a working relationship with the CDC Bioterrorism Rapid Response Advanced Technology Laboratory was established, and evaluation of multiplexed immunoassays for several real bioagents occurred at the CDC facility in Atlanta. During this exercise, multiplex immunoassays for 6 biothreat agents were shown to be comparable in sensitivity and selectivity to the CDC standard. In addition, minimal cross reactivity with 25 different *Ba* and 20 *Yp* isolates was also demonstrated. Extension of this agent set and further validation studies are planned for FY03.

Field testing

In April of 2002, the APDS unit was fielded at the Harry Reid Center for Environmental Studies at UNLV. The purpose of this study was to evaluate the performance of the fully autonomous system with an aerosolized *Bacillus anthrasis* simulant (*Bacillus globigii*). This field test was set as a first step towards functional testing of the system using real agents at Dugway Proving Grounds later in the year. The results demonstrated that APDS sensitivity was comparable to the gold standard benchtop immunoassays. There was good intersystem agreement between the external and in-chamber systems, with little or no cross-reactivity between assays. Based on these results, a field deployment for live agent testing at Dugway Proving Grounds was conducted in September of 2002. Aersolized, live isolates of *Bacillus anthrasis* and *Yersinia pestis* were released and detected using the APDS system. Successful completion of this field trial benchmarked the first, fully autonomous detection of live agents using the multiplexed immunoassay approach.

Development of flow-through PCR for the APDS-II

In parallel to deployment of a real agent immunoassay system, characterization and further optimization of the confirmatory, PCR unit occurred in FY02. This prototype flow-through PCR module consists of an LLNL-designed, silicon-machined, thermocycler that is mounted in-line with our sample preparation unit. The use of silicon components allows thermocycling to proceed very rapidly (less than 1 minute per heat/cool cycle). The thermocycler contains appropriate light sources and detectors to perform real-time TaqMan assays. Less than 3 copy detection was demonstrated with a >95% amplification efficiency. DNA titration curves in triplicate indicated that the design is extremely stable and uniform. Decontamination studies also proved that the chamber was reusable within five minutes of the previous run.

Technology Transfer

LLNL has licensed the current version of APDS to BAE Systems. It is reasonable to expect that transition of this platform will occur in phases, as new capabilities become available. As part of this effort, BAE has already significantly reduced the size and increased the ruggedness of the multiplexed detector component.

Future Outlook

The first version of APDS-II was completed by the end of FY02, and is capable of performing real-agent, automated multiplex immunoassays. The full APDS-II system will include automated multiplex immunoassays followed by a confirmatory, nucleic acid based test. This system has been licensed to a commercial partner and a cooperative research agreement is being put into place. APDS-III corresponds to the final APDS prototype and will incorporate a bead-based, multiplex nucleic acid detection capability. This version is slated for completion at end of FY04.